Role of KITENIN in Progression and Metastasis of SCC

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BACKGROUND

The expression of KAI1 (a metastatic suppressor gene) in cancer cells results in reduced cell motility and invasiveness. A cDNA clone of VANGL1, a member of the tetraspan protein family that specifically interacts with the COOH-terminal cytoplasm domain of KAI1, was isolated and renamed KITENIN (KAI1 COOH-terminal interacting tetraspanin).

The purpose of this study was to investigate the role of KITENIN on the progression and metastasis of transfected squamous cell carcinoma using in vivo and in vitro experiments.

MATERIALS AND METHODS

Locally advanced squamous cell carcinoma tissues from five patients were obtained for investigation of KITENIN expression. Malignant tumors, normal adjacent mucosa tissues, metastatic lymph nodes, and non-metastatic lymph nodes were collected.

KITENIN or vector only (control) was transfected into SCC (squamous cell carcinoma) VII, a mouse squamous cell carcinoma cell line, using FuGENE 6. An in vitro assay (invasion, migration, and proliferation) for KITENIN and the vector-transfected group was studied.

The KITENIN or vector-transfected SCC VII cells were injected subcutaneously into 12 C57Bl/6J syngeneic mice (6 mice for each group). The tumor size was measured daily for 4 weeks. During the fifth week after injection, the presence of metastasis in the lung and liver tissue was evaluated for each mouse with a tumor mass on the back, the tissues were assessed by gross and microscopic examination.

To investigate the relationship between KITENIN and AP-1 axis in squamous cancer cells, some well-known AP-1 target genes were compared between the KITENIN- and empty vector-transfected SCC VII cells, such as cyclin D1, MMP-1, COX-2, and CD44.

RESULTS

Increased KITENIN expression in the tumor and metastatic lymph nodes from HNSCC patients.

Increased expression of AP-1 target genes in KITENIN-transfected squamous cancer cells.

CONCLUSION

KITENIN-overexpressing SCC VII cells demonstrated increased invasiveness, migration, and proliferation in vitro and increased tumorigenicity and lung metastasis in vivo, compared with the vector-transfected cells. Therefore, an antisense KITENIN strategy may be a useful method to inhibit metastasis in squamous cell carcinoma.

**Fig. 1** Expression of KITENIN in resected tissues from HNSCC patients.

**Fig. 2** KITENIN enhances cell invasion, migration, and proliferation of SCC VII cells. (A) Invasion assay of SCC VII cells using fibronectin as a chemotactictractant. Invading cells was larger in the KITENIN-transfected SCC VII cells than in the empty vector-transfected SCC VII cells (left). Stained invading cells were counted and are represented as a bar graph between groups (right) (mean ± SEM, n = 3, **p<0.01). (B) The effects of the overexpressed KITENIN on cell migration. The empty vector- or KITENIN-transfected SCC VII cells were subjected to a wound healing assay (left), and cell migration are displayed as relative healing distances measured in six random sites (right). Values are mean ± SEM for three independent experiments (**p<0.01). Cell migration was markedly increased in the KITENIN-transfected SCC VII cells. (C) The effects of the overexpressed KITENIN on cell proliferation. The absorbance indicating proliferating viable cells was higher in the KITENIN-transfected SCC VII cells (n = 3, mean ± SEM, *p<0.05).

**Fig. 3** The effects of the overexpressed KITENIN on in vivo tumor growth and distant metastasis in a syngeneic mouse squamous tumor model.

(A) Transfected SCC VII cells were injected subcutaneously into the right flank of syngeneic mice and tumor size was measured daily. Tumor growths were increased in the KITENIN-transfected SCC VII-injected C57Bl/6J syngeneic mice compared to the vector-transfected SCC VII-injected mice (n = 6, mean ± SEM, *p<0.01). (B) H & E stained metastasized lung tissue from the KITENIN-transfected SCC VII-injected C57Bl/6J syngeneic mice. The representative tumor cell nest invading the pleura (a thin arrow) and alveoli (a thick arrow) was shown (×40). Dyskeratosis, frequent mitosis, coarse chromatin, and nuclear pleomorphism were found in a high-power field view of pleural metastatic tissue (inset, H & E stain, ×400).

**Fig. 4** Expression of AP-1 target genes in the KITENIN-transfected SCC VII cells. Expression of cyclin D1, MMP-1, COX-2, and CD44 were higher in the KITENIN-transfected SCC VII cells.